Supporting Information

Understanding laccase/HBT-catalyzed grass delignification at the molecular level

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Laccase activity determination (ABTS assay)

The activity of the commercial laccase preparation was determined spectrophotometrically by oxidation of ABTS. Hereto, a 1 mL quartz cuvette was filled with 0.5 mM ABTS in a sodium acetate buffer (pH 5, 100 mM). A solution of laccase was added and the increase in absorbance at 420 nm was measured over time (ε = 36,000 M^{-1} cm^{-1}). The laccase activity was expressed in units (1 U = 1 µmol ABTS oxidized per minute).

Estimation of total laccase activity in laccase/HBT treatments

Incubations at pH 6 were performed both at equal laccase concentration and at equal laccase activity, as compared to pH 4. In order to determine the required laccase concentration at pH 6 to obtain equal laccase activity as compared to pH 4, we determined: (i) the ratio (pH 4 to pH 6) of initial laccase activity toward HBT, and (ii) the ratio (pH 4 to pH 6) of integrated residual laccase activity in incubations with HBT and milled wheat straw (MWS). The product of these two ratios was used to determine the required laccase concentration at pH 6, to obtain a similar overall activity as in the incubation with 50 U of laccase at pH 4.

Determination of laccase activity toward HBT

Relative laccase activity (pH 4 compared to pH 6) toward HBT was determined by using two methods: oxygen consumption and UV/VIS spectrophotometry. The average between the two measurements was used in further calculations.

For the oxygen consumption measurements were performed by using an Oxytherm System (Hansatech Instruments, Pentney, UK). Hereto, 1 mL of a 78 mM HBT solution in 200 mM citrate buffer at pH 4 or 6 was added to the measuring cell, and the sample was allowed to equilibrate at 40 ºC under magnetic stirring (75 rpm) for approximately 1 h. Next, 0.12 U laccase was added and the oxygen concentration was monitored over time. The slopes of the oxygen consumption measurements were then corrected using blank measurements (without laccase) and used to estimate the ratio of laccase activity at pH 4 relative to pH 6 (Table S1).

For UV/VIS spectrophotometry, 1 mL aliquots of the same HBT solution as described above were used at 40 ºC. Laccase (0.23 U) was added, and spectra (300-600 nm) were recorded every minute on a Shimadzu UV1800 spectrophotometer equipped with a CPS-240A temperature controller. The absorbance at 420 nm was plotted over time, and the slope was used to estimate the ratio of laccase activity at pH 4 relative to pH 6 (Table S1).

Determination of integrated residual laccase activity in incubations with HBT and MWS

HBT solutions (250 µL, 78 mM) at either pH 4 or pH 6 in a 200 mM citrate buffer were added to 650 µL Eppendorf tubes. MWS was suspended at a 6% (w/w) concentration and laccase was added at a dose of 33 U g^{-1} MWS. Immediately after enzyme addition, a 10 µL sample was withdrawn and stored at 4 ºC. Next, the tubes were incubated in a thermomixer (40 ºC; 1,100 rpm). Samples of 10 µL were taken after 1, 2, 4.5, 7 and 21 h, and stored at 4 ºC. To determine the laccase activity, samples were diluted with sodium acetate buffer (100 mM, pH 5), vortexed, centrifuged, and the activity of the supernatant was determined by using the ABTS assay (see page S2).[1] The residual laccase activity (RA, %) was plotted versus time and a first-order exponential decay was fitted to the data according to the equation:

$$RA = e^{-kt}$$

where k (s^{-1}) is the inactivation rate constant an t (s) is the time. The functions were then integrated to obtain the areas under the curves, which are a measure for the total laccase activity in a 21 h incubation. The ratio between the areas at pH 4 and pH 6 was used to compensate for differences in laccase stability (Table S1).

Table S1. Ratio (pH 4 to pH 6) of laccase activity and of integrated residual laccase activity.

	Ratio pH 4 to pH 6
Activity toward HBT (O_2 consumption)	5.9 ± 0.71
Activity toward HBT (spectrophotometry)	7.4 ± 0.93
Integrated residual activity	0.38 ± 0.05
Overall activity	2.5

Based on the results displayed in Table S1, incubations at pH 6 were performed with both 50 and 125 U g^{-1} MWS/MCS.

Flash chromatography purification of WECEL and WEL fractions

The Rovabio® Advance treated and pooled WECEL and WEL fractions were injected on a Grace Reveleris Flash chromatography system (Grace, Columbia, MD, USA), equipped with a Reveleris C18 RP 40 g cartridge (particle size 40 μm). Eluents used were water (A) and acetonitrile (B), both containing 1% (v/v) formic acid. The flow rate was 30 mL min^{-1}. UV absorbance at 280 and 310 nm and evaporative light scattering detector (ELSD) were used to monitor the elution of compounds. The following elution program was used: 0–3 min, isocratic at 0% B; 3–6.5 min, linear gradient to 14 % B; 6.5–12.5 min, isocratic at 14% B; 12.5–26 min, linear gradient to 68% B; 26–29.2 min, linear gradient to 100% B; 29.2–37.2 min, isocratic at 100% B; 37.2–39.2 min, linear gradient to 0% B; 39.2–43.2 min, isocratic at 0% B. The eluent was collected from 16.5-39.2 min, after which it was concentrated under reduced pressure at 30 °C, and lyophilized to obtain purified water extractable lignin (WELpure).

For WECEL purification, a slightly different elution program was used with a flow rate of 40 mL min^{-1}: 0–2.9 min, isocratic at 0% B; 2.9–12.9 min, linear gradient to 20% B; 12.9–20.9 min, linear gradient to 68% B; 20.9–23.4 min, linear gradient to 100% B; 23.1–29.1 min, isocratic at 100% B; 29.1–31.1 min, linear gradient to 0% B; 31.1–34 min, isocratic at 0% B. The eluent was collected from 12.5–31 min, after which it was concentrated under reduced pressure at 30 °C, and lyophilized to obtain purified water extractable cellulolytic enzyme lignin (WECELpure).

Quantitative py-GC-MS with ^{13}C-lignin as internal standard

Quantitative pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) was performed by using an EGA/PY-3030D Multi-shot pyrolyzer (Frontier Laboratories, New Ulm, MN, USA) equipped with an AS-1020E Autoshot auto-sampler as described previously.[2] The pyrolyzer was coupled to a GC-MS system consisting of a Trace GC (Thermo Scientific, Waltham, MA, USA) equipped with a DB-1701 fused-silica capillary column (30 m × 0.25 mm i.d. 0.25 μm film thickness), of which the first meter is employed as pre-column, and an Exactive Orbitrap Mass Spectrometer (Thermo Scientific). Samples were weighed using an XP6 excellence-plus microbalance (Mettler Toledo, Columbus, OH, USA). Sample masses were used roughly according to their estimated lignin content: ~60 μg for CEL, ~40 μg for WELpure, ~40 μg for WECELpure, and ~80 μg for all other samples. To all samples, 10 μL of a ^{13}C-labeled wheat straw lignin isolate solution (1 mg mL^{-1} in CHCl3:EtOH 50:50 v/v) was added as an internal standard. Samples were then air dried at room temperature prior to analysis. Pyrolysis was performed at 500 °C for 1 min with an interface temperature of 300 °C. Pyrolysis products were injected on the column via split/splitless injection at 250 ◦C with a split ratio of 1:133 and helium was used as carrier gas with constant flow at 1.5 mL min^{-1}. The GC oven was programmed from 70 °C (2 min) to 270 °C at 5 °C min^{-1} and held at 270 °C for 15 min. MS detection was used with EI at 70 eV, a source temperature of 275 °C, a resolution of 60,000, AGC target at 10^6, maximum IT at 'auto' and a scan range of *m/z* 35-550. Fifty one lignin pyrolysis compounds were identified by comparing retention time and mass spectrum with literature (Table S2).[2]

Pyrograms were processed by Tracefinder 4.0 software (Thermo Scientific). For each compound, the most abundant ion was selected and automatically integrated using ICIS peak integration with optimized settings per compound. Manual corrections were only performed when irregular peak shapes were observed that led to erroneous peak integration with automated integration. From the processed data, lignin contents and relative abundances of lignin-derived pyrolysis products were calculated as described previously.[3] For absolute lignin quantification, all lignin-derived pyrolysis products were included in the processing. In case pyrolysis data were used to fingerprint structural changes in lignin, two pyrolysis products were excluded from processing: 4-vinylphenol (4-VP) and 4-vinylguaiacol (4-

VG). This was mainly done to improve the comparability between the py-GC-MS results and the results from HSQC NMR experiments. The rationale behind this, is that in HSQC NMR analysis, FA and *p*CA can be distinguished from 'core lignin' units, while pyrolysis of hydroxycinnamic acids and lignin interunit linkages both yield vinyl products.[4, 5]

In more detail, for relative quantification of C_α-oxidation and intact interunit linkages in HSQC NMR, only S, S_{ox}, G, G_{ox} and H-units were taken into account. For comparative purposes, the origin of the vinyl products in py-GC-MS based relative quantifications should be dealt with. Although 4-VP can also be formed from pyrolysis of H-units, the abundance of H-units is low compared to that of pCA (especially in corn stover). It can therefore be fairly assumed that 4-VP mainly originates from pyrolysis of pCA, and that exclusion from 4-VP increases the comparability between HSQC and py-GC-MS results. The formed 4-VG, however, can originate from both FA and G-units, which are both highly abundant in WS and CS lignin. Therefore, neither inclusion nor exclusion of 4-VG would result in a perfect comparability between both techniques. We decided to exclude 4-VG from data comparison. For py-GC-MS based estimation of the *p*CA content, the relative abundance of 4-VP was taken from the total abundance of lignin-derived pyrolysis products (i.e. including 4-VP and 4-VG) and reported separately (Table S18).

Table S2. Identity and structural classification of lignin-derived pyrolysis products by ^{13}C-IS py-GC-MS.

#	Compound	Rt (min)	Structural feature	Side-chain length	Mw ^{12}C (Da)	Quan ion ^{12}C [M-e$^-$]	Mw ^{13}C (Da)	Quan ion ^{13}C [M-e$^-$]
1	phenol	9.79	H, unsub.	—	94	94.041320	100	100.06145
2	guaiacol	10.03	G, unsub.	—	124	124.05188	131	115.04853
3	2-methylphenol	11.03	H, methyl	1	108	108.05698	115	115.08045
4	4-methylphenol (+3-MP)	12.00	H, methyl	1	108	107.04914	115	114.07263
5	4-methylguaiacol	12.71	G, methyl	1	138	138.06753	146	146.09437
6	2,4-dimethylphenol	13.18	H, methyl	1	122	107.04914	130	114.07263
7	4-ethylphenol	14.25	H, misc.	2	122	107.04914	130	114.07263
8	4-ethylguaiacol	14.83	G, misc.	2	152	137.05971	161	145.08654
9	4-vinylguaiacol[a]	16.29	G, vinyl	2	150	150.06753	159	159.09754
10	4-vinylphenol[a]	16.46	H, vinyl	2	120	120.05697	128	128.08381
11	eugenol	16.89	G, misc.	3	164	164.08318	174	174.11673
12	4-propylguaiacol	16.99	G, misc.	3	166	137.05971	175	145.08654
13	syringol	17.64	S, unsub.	—	154	154.06245	162	162.08928
14	*cis*-isoeugenol	18.25	G, misc.	3	164	164.08318	174	174.11673
15	4-propenylphenol	19.24	H, misc.	3	134	133.06479	143	142.09498
16	*trans*-isoeugenol	19.50	G, misc.	3	164	164.08318	174	174.11673
17	4-methylsyringol	19.86	S, methyl	3	168	168.07810	177	177.10829
18	vanillin	19.99	G, C_α-O	3	152	151.03897	160	159.06581
19	4-propyneguaiacol	20.23	G, misc.	3	162	162.06753	172	172.10108
20	4-alleneguaiacol	20.49	G, misc.	3	162	162.06753	172	172.10108
21	homovanillin	21.44	G, C_β-O	2	166	137.05971	175	145.08654
22	4-ethylsyringol	21.58	S, misc.	2	182	167.07022	192	176.10046
23	vanillic acid methyl ester	21.82	G, C_α-O	1	182	182.05736	191	191.08766
24	acetovanillone	21.89	G, C_α-O	2	166	151.03897	175	159.06581
25	4-hydroxybenzaldehyde	22.76	H, C_α-O	1	122	121.02848	129	128.05189
26	4-vinylsyringol	22.90	S, vinyl	2	180	180.07810	190	190.11164
27	guaiacylacetone	23.10	G, C_β-O	3	180	137.05971	190	145.08654
28	4-allylsyringol	23.31	S, misc.	3	194	194.09373	205	205.13065
29	propiovanillone	23.79	G, C_α-O	3	180	151.03897	190	159.06581
30	guaiacyl vinyl ketone	24.09	G, C_α-O	3	178	151.03897	188	159.06581
31	guaiacyl diketone	24.32	G, C_α-O, C_β-O	3	194	151.03897	204	159.06581
32	*cis*-4-propenylsyringol	24.43	S, misc.	3	194	194.09373	205	205.13065
33	4-propynesyringol	25.06	S, misc.	3	192	192.07810	203	203.11500
34	4-allenesyringol	25.27	S, misc.	3	192	192.07810	203	203.11500
35	*trans*-4-propenylsyringol	25.72	S, misc.	3	194	194.09373	205	205.13065
36	dihydroconiferyl alcohol	25.81	S, C_γ-O	3	182	137.05971	192	145.08654
37	syringaldehyde	26.34	S, C_α-O	1	182	182.05736	191	191.08755
38	*cis*-coniferyl-alcohol	26.42	G, C_γ-O	3	180	137.05971	190	145.08654
39	homosyringaldehyde	27.32	S, C_β-O	2	196	167.07027	206	176.10046
40	syringic acid methyl ester	27.66	S, C_α-O	1	212	212.06793	222	222.10147
41	acetosyringone	27.76	S, C_α-O	2	196	181.04954	206	190.07973
42	*trans*-coniferyl alcohol	28.11	G, C_γ-O	3	180	137.05971	190	145.08654
43	*trans*-coniferaldehyde	28.50	G, C_γ-O	3	178	147.04406	188	156.07425
44	syringylacetone	28.68	S, C_β-O	3	210	167.07027	221	176.10046
45	propiosyringone	29.29	S, C_α-O	3	210	181.04954	221	190.07973
46	syringyl diketone	29.43	S, C_α-O, C_β-O	3	224	181.04954	235	190.07973
47	syringyl vinyl ketone	29.57	S, C_α-O	3	208	181.04954	219	190.07973
48	dihydrosinapyl alcohol	31.13	G, C_γ-O	3	212	168.07841	223	177.10829
49	*cis*-sinapyl alcohol	31.63	S, C_γ-O	3	210	167.07027	221	176.10046
50	*trans*-sinapyl alcohol	33.31	S, C_γ-O	3	210	167.07027	221	176.10046
51	*trans*-sinapaldehyde	33.54	S, C_γ-O	3	208	208.07301	219	219.10994

[a] 4-vinylphenol and 4-vinylguaiacol were excluded from all calculations. The relative abundance of 4-vinylphenol is reported separately in Table S18.

NMR spectroscopy

Sample preparation

NMR spectroscopy of CEL samples was performed in gel-state, based on a previously described protocol.[6] Hereto, approximately 40 mg CEL was swollen with 500 µL (for CS) or 650 µL (for WS) deuterated dimethyl sulfoxide (DMSO-d_6) in a 5 mm NMR tube, followed by sonication for 1-5 h to obtain a homogeneous gel. WECEL$_{pure}$ samples (15-60 mg) were dissolved in 450 µL DMSO-d_6, vortexed, and transferred to a 5 mm NMR tube. For WEL$_{pure}$ samples, solutions of 15-45 mg in 200 µL DMSO-d_6 were prepared, vortexed, and transferred to a 5 mm Shigemi NMR microtube.

HSQC NMR analysis

HSQC experiments were performed based on previously reported methods.[6] Spectra were recorded at 25 °C on a Bruker AVANCE III 600 MHz NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 5 mm cryoprobe. Spectra were recorded using a standard Bruker pulse sequence 'hsqcetgpsisp2.2'. The spectral widths were 0-12 ppm (7,200 Hz) for the ^{1}H dimension and 0-200 ppm (30,000 Hz) for the ^{13}C dimension. Sixteen scans were acquired with a relaxation time of 1 s and a FID size of 2018 in the ^{1}H dimension, and 400 the ^{13}C dimension. For $^{1}J_{CH}$ 145 Hz was used.

Data was processed with Bruker TopSpin version 4.0.5. DMSO-*d6* (δC 39.5 ppm; δH 2.49 ppm) was used to calibrate the chemical shifts. Processing was performed by Gaussian apodization, and a squared cosine function in the ^{1}H and ^{13}C dimensions, respectively. For the different sample fractions slightly different values for LB and GB were used, WELpure, and WECELpure: LB = -0.2 and GB = 0.001; WS-CEL LB = -0.20 and GB = 0.0005; CS-CEL LB = -0.30 and GB = 0.0005. LPfr linear prediction in F1 of 32 points was performed. Prior to Fourier transformation, the ^{13}C dimension was zero filled up to 1024 points.

HSQC correlations were assigned in accordance to literature (Table S3).[7-11] Semi-quantitative volume integration was performed as previously described by Del Río et al.,[8] on a single zoom level within each sample.

The abundances of β-*O*-4' substructures and the cleavage products (DHPV, DHPS, HPV, HPS) were determined using their C_{β}-H_{β} correlations. For phenylcoumaran (B) and resinol (C) substructures C_{α}-H_{α}

correlations were used. The signals of HPV+HPS (L) and resinol (C) were logically halved. $S_{2,6}$, G_2, and $H_{2,6}$ signals were used for S, G, and H units, respectively, where S and H integrals were halved as well. Abundances of oxidized analogues were estimated in a similar manner. Tricin, *p*CA, and FA were determined from their respective $T_{2',6'}$, $pCA_{2,6}$, and FA_2 signals. $H_{2,6}$ integrals were corrected for the overlapping phenylalanine cross peak ($PHE_{3,5}$) by subtraction of the isolated $PHE_{2,6}$ cross-peak integral.[12] Amounts were calculated relative to the total aromatic lignin subunits ($H + G + G_{ox} + S + S_{ox} = 100$).

HMBC experiments

HMBC experiments were performed with the following WS fractions: $WECEL_{pure}$ (pH 4, control), $WECEL_{pure}$ (pH 4, 50 U g^{-1}) and WEL_{pure} (pH 6, 125 U g^{-1}). Hereto, 20, 32 and 49 mg of the fractions, respectively, were dissolved in 200 µL DMSO-d_6, vortexed, and transferred into Shigemi NMR microtubes. Experiments were performed similar to the HSQC experiments, using the standard Bruker pulse program 'hmbcgplpndqf'. Sixteen dummy scans and 88 scans were acquired with an evolution period of 53 ms for long range coupling. The FID size was 8192 in the ^{1}H dimension, and 400 the ^{13}C dimension. Processing used Gaussian apodization (LB=-5, GB=0.8), and a squared cosine function (SSB=1) in the ^{1}H and ^{13}C, respectively.

Table S3.Assignments of the lignin $^{1}H/^{13}C$ correlation signals in HSQC NMR spectra. Assignments are based on literature.[7-12] t = tentative annotation.

Label	δ_C/δ_H (ppm)	Assignment
E_β	40.3/3.08	C_β-H_β of HPV and HPS, i.e. β-*O* cleaved β-*O*-4' linkage
F-$_{OCH3}$	51.6/3.60	C-H in methoxyl groups of cyclohexadienone ketals (t)
C_β	53.0/3.43	C_β-H_β in resinol substructures
B_β	53.6/3.05	C_β-H_β in phenylcoumaran substructures
-OCH_3	55.6/3.73	C-H in methoxyl groups
E_γ	56.9/3.75	C_γ-H_γ in HPV and HPS, i.e. β-*O* cleaved β-*O*-4' linkage
A_γ	59.6/3.4 and 3.7	C_γ-H_γ in β-*O*-4' substructures
J_γ	61.4/4.09	C_γ-H_γ in cinnamyl alcohol end groups
A'_γ/A''_γ	62.3/4.02	C_γ-H_γ in C_α oxidized β-*O*-4' substructures
B_γ	62.6/3.67	C_γ-H_γ in phenylcoumaran substructures
D_γ	64.0/3.64	C_γ-H_γ in DHPV and DHPS, i.e. *O*-4' cleaved β-*O*-4' linkage
$A_{\gamma(ac)}$	64.2/4.1 and 4.3	C_γ-H_γ in acylated β-*O*-4' substructures
A_α (G)	70.9/4.71	C_α-H_α in β-*O*-4' substructures linked to a G-unit
C_γ	71.0/3.79 and 4.16	C_γ-H_γ in resinol substructures
A_α (S)	71.8/4.81	C_α-H_α in β-*O*-4' substructures linked to a S-unit
D_β	73.8/4.98	C_β-H_β of DHPV and DHPS, i.e. *O*-4' cleaved β-*O*-4' linkage
A''_β (G)	81.2/5.88	C_β-H_β in C_α oxidized β-*O*-4' substructures linked to a C_α-
A'_β (S)	83.2/5.18	C_β-H_β in C_α oxidized β-*O*-4' substructures linked to a S-unit
A''_β (S)	83.2/5.57	C_β-H_β in C_α oxidized β-*O*-4' substructures linked to a C_α-
A_β (H)	83./4.49	C_β-H_β in β-*O*-4' substructures linked to a H-unit
A_β (G)	83.5/4.27	C_β-H_β in β-*O*-4' substructures linked to a G-unit
C_α	84.9/4.64	C_α-H_α in resinol substructures
A_β ($S_{erythro}$)	85.9/4.09	C_β-H_β in β-*O*-4' substructures linked to an $S_{erythro}$-unit
A_β (T)	86.2/4.36; 86.7/4.26	C_β-H_β in β-*O*-4' substructures linked to a T-unit*
A_β (S_{threo})	86.9/3.97	C_β-H_β in β-*O*-4' substructures linked to an S_{threo}-unit
B_α	86.9/5.43; 87.6/5.54	C_α-H_α in phenylcoumaran substructures
T_8	94.1/6.57	C_8-H_8 in tricin
T_6	98.8/6.21	C_6-H_6 in tricin
$S_{2,6}$	103.9/6.69	C_2-H_2 and C_6-H_6 in S-unit
$T_{2',6'}$	104.0/7.31	C_2'-H_2' and C_6'-H_6'in tricin
T_3	104.6/7.04	C_3-H_3 in tricin
$Sox_{2,6}$ (carbonyl)	106.4/7.30	C_2-H_2 and C_6-H_6 in C_α-oxidized (C_α=O) S-unit
$Sox_{2,6}$ (acid)	106.5/7.19	C_2-H_2 and C_6-H6 in C_α-oxidized (C_αOOH) S-unit
G_2	110.8/6.96	C_2-H_2 in G-unit
FA_2	110.9/7.34	C_2-H_2 in ferulate
Gox_2	111.4/7.51; 112.4/7.45	C_2-H_2 in C_α-oxidized G-unit
$H_{3,5}/FA_5$	114.6/6.70	C_3-H_3 and C_5-H_5 in H-unit, C_5-H_5 in FA
G_5/G_6	114.9/6.76, 118.7/6.81	C_5-H_5 and C_6-H_6 in G-unit, C_3-H_3
Gox_5	115.0/6.80	C_5-H_5 in C_α-oxidized G-unit
$pCA_{3,5}$	115.0/6.75	C_5-H_5 of *p*CA
FA_β/pCA_β	115.3/6.33	C_β-H_β in ferulate/*p*-coumarate
FA_6	122.5/7.09	C_6-H_6 in ferulate
Gox_6	122.7/7.51	C_6-H_6 in C_α-oxidized G-unit
Phe_4	126.3/7.18	C_4-H_4 in phenylalanine
$H_{2,6}/Phe_{3,5}$	127.7/7.18	C_2-H_2 and C_6-H_6 in H-units, C_3-H_3 and C_5-H_5 in phenylalanine
$Phe_{2,6}$	129.0/7.21	C_2-H_2 and C_6-H_6 in phenylalanine
$Tyr_{2,6}$	129.8/7.00	C_6-H_6 in C_α-oxidized G-unit
$PHE_{2,6}$	128.0/7.21	C_2-H_2 and C_6-H_6 in phenylalanine
$pCA_{2,6}$	130.1/7.50	C_2-H_2 and C_6-H_6 in *p*-coumarate
FA_α/pCA_α	144.2/7.49	C_α-H_α in ferulate/*p*-coumarate

* Or electron-withdrawing moieties other than tricin

RP-UHPLC-PDA-MS

High resolution RP-UHPLC-PDA-MS analysis was performed as described in a previous study,[1] using water (A) and acetonitrile (B) as eluents, both containing 0.1% formic acid. An adapted elution gradient was used: From 0-1.5 min at 5% B (isocratic), 1.5-35 min from 5 to 60% B (linear gradient), 35 to 41.2 min from 60 to 100% B (linear gradient), 41.2-45.7 min at 100% B (isocratic), 45.7-46.2 min from 100 to 5% B (linear gradient) and 46.2-50 min at 5% B (isocratic). The capillary temperature was 254 °C; the probe heater temperature was 408 °C; S-lens RF level was 50 and the source voltages were 3.5 and 2.5 kV in positive and negative ionization mode, respectively. Nitrogen was used as sheath gas (46.6 arbitrary units) and auxiliary gas (10.8 arbitrary units). All other settings were the same as described in Hilgers et al.[1]

Table S4 Recoveries (%) of residues of wheat straw and corn stover during extraction, ball milling and washing relative to the starting material (dry matter) of each step. N.D. = Not determined.

Step	Wheat straw	Corn stover
Soxhlet extraction	98	N.D.
Ball-milling	92	93
Washing (water)	80	84

Table S5 Klason lignin determination of MWS and its RES fractions after control and laccase/HBT incubations (24h) at pH 4. Acid-soluble lignin could not be determined, due to the interfering UV absorption of HBT and its degradation products.

	MWS	Control	Laccase/HBT
Acid-insoluble lignin (AIL) (%)[a]	21.0 ± 0.6	20.1 ± 0.7	15.0 ± 0.4
Klason lignin (mg)[a]	-	292 ± 10	216 ± 7
Ash (% of AIL)	14.7 ± 1.0	16.3 ± 1.8	16.3 ± 1.5
Protein (% of AIL)[b]	3.2 ± 0.0	3.7 ± 0.1	8.1 ± 1.0
Delignification (%)		7.4	31.7

[a] Corrected for ash and protein content.

[b] As determined by using the Dumas method with N-to-protein ratio 6.25, assuming that all nitrogen originates from protein

Effects of laccase/HBT treatment on lignin quantification by using Klason and py-GC-MS methodologies

The most widely applied method for lignin quantification is the Klason lignin determination method, which relies on gravimetric analysis of samples after a hydrolytic treatment with sulfuric acid (including correction for ash and protein content). Although this method gives a fairly accurate estimation of the lignin content of untreated biomass, the presence of HBT, either in free form or grafted, may heavily interfere with the lignin determination. In our case, HBT and its degradation product BT were, despite extensive washing, still present in the residue (see Table S6). During the Klason method, free HBT and BT are expected to end up in the acid-soluble fraction. Since these products show absorbance at 205 nm, no reliable acid-soluble lignin content could be determined in this study. For grafted HBT it is unclear how it influences the Klason lignin determination. If grafted HBT is cleaved off during the sulfuric acid treatment, it will also end up in the acid-soluble fraction, but if it 'survives' the sulfuric acid treatment, it will be measured as acid-insoluble lignin, and thereby result in an overestimation of the lignin content. The presence of grafted HBT will also interfere with the protein correction, which is based on nitrogen content.

To overcome the major drawback of the Klason method (i.e. poor selectivity for lignin), recently, a py-GC-MS based lignin quantification method was developed.[2] In this method, lignin-derived pyrolysis products are measured exclusively, and quantification is performed using a ^{13}C-labeled wheat straw lignin isolate as internal standard. The method has been shown to accurately quantify lignin in several grasses, amongst which wheat straw and corn stover. Nevertheless, it is possible that reactions such as (re)polymerization and grafting resulted in substructures that are considerably more resistant against pyrolysis than those originally present in the substrate and internal standard.[13, 14] These substructures may, therefore, have accumulated in the pyrolysis residue or may have been released as (dimeric) products that were not quantified in the used method. Consequently, the formation of such substructures likely resulted in an underestimation of the lignin content of the laccase/HBT treated samples, and thus in an overestimation of delignification.

Although we cannot prove that py-GC-MS is a more accurate quantification method for laccase/HBT samples, we chose to use this method for quantification of lignin since it also provides useful information on the lignin structure and it can also be used on soluble fractions.

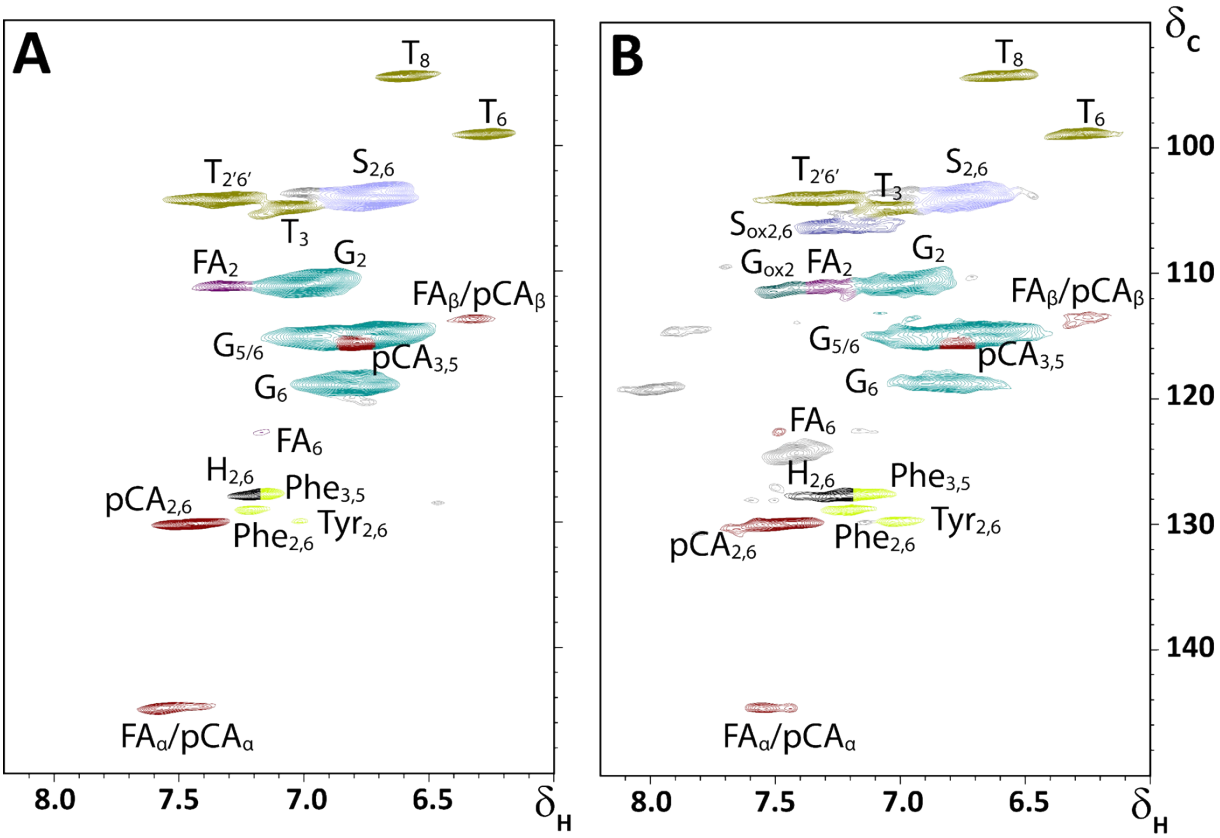

Fig. S1 Aromatic regions of the HSQC spectra obtained from CEL fractions of MWS treated with laccase/HBT at pH 4 (B) and the corresponding control (A). HSQC analyses of other CEL fractions showed the same peaks with different intensities. Only processed data from these spectra are shown (in other Figures and Tables). Example spectra of $WECEL_{pure}$ and WEL_{pure} fractions are shown in Fig. 5.

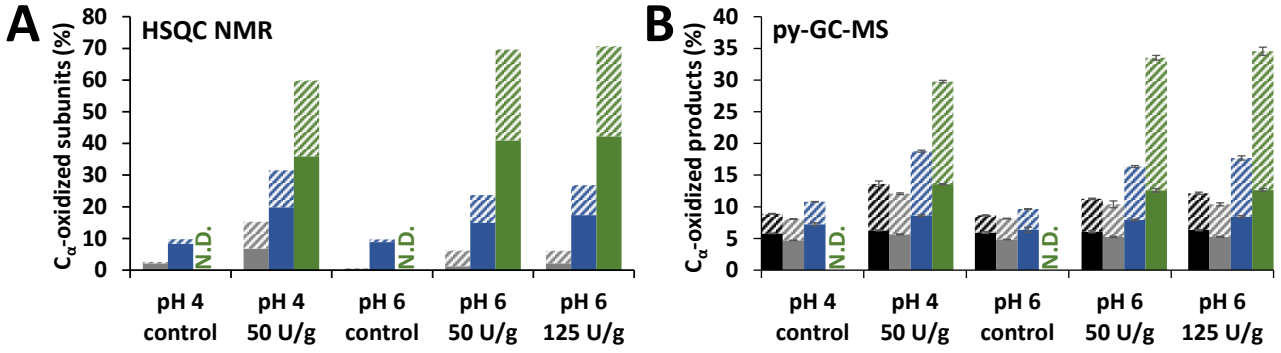

Fig. S2 Relative abundance of C_α-oxidized structures as determined by HSQC NMR (A) and estimated based on py-GC-MS (B) in RES (black), CEL (grey), $WECEL_{pure}$ (blue) and WEL_{pure} (green) fractions of laccase/HBT treated MCS and controls. Solid and striped bars correspond to G and S units, respectively. Because of their low abundance, H-units are not included. Error bars in B represent the standard deviation of two treatment duplicates and two analytical duplicates. N.D. = Not determined.

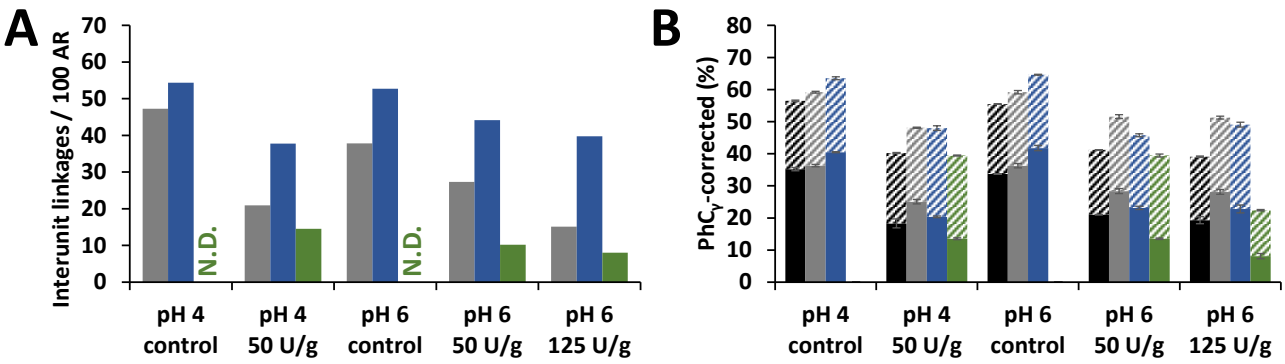

Fig. S3 HSQC NMR based (A) and py-GC-MS based (B) estimation of intact interunit linkages in RES (black), CEL (grey), $WECEL_{pure}$ (blue) and WEL_{pure} (green) fractions of laccase/HBT treated MCS and controls. In B, solid and striped bars refer to G and S units, respectively. Error bars in B represent the standard deviation of two treatment duplicates and two analytical duplicates. N.D. = Not determined.

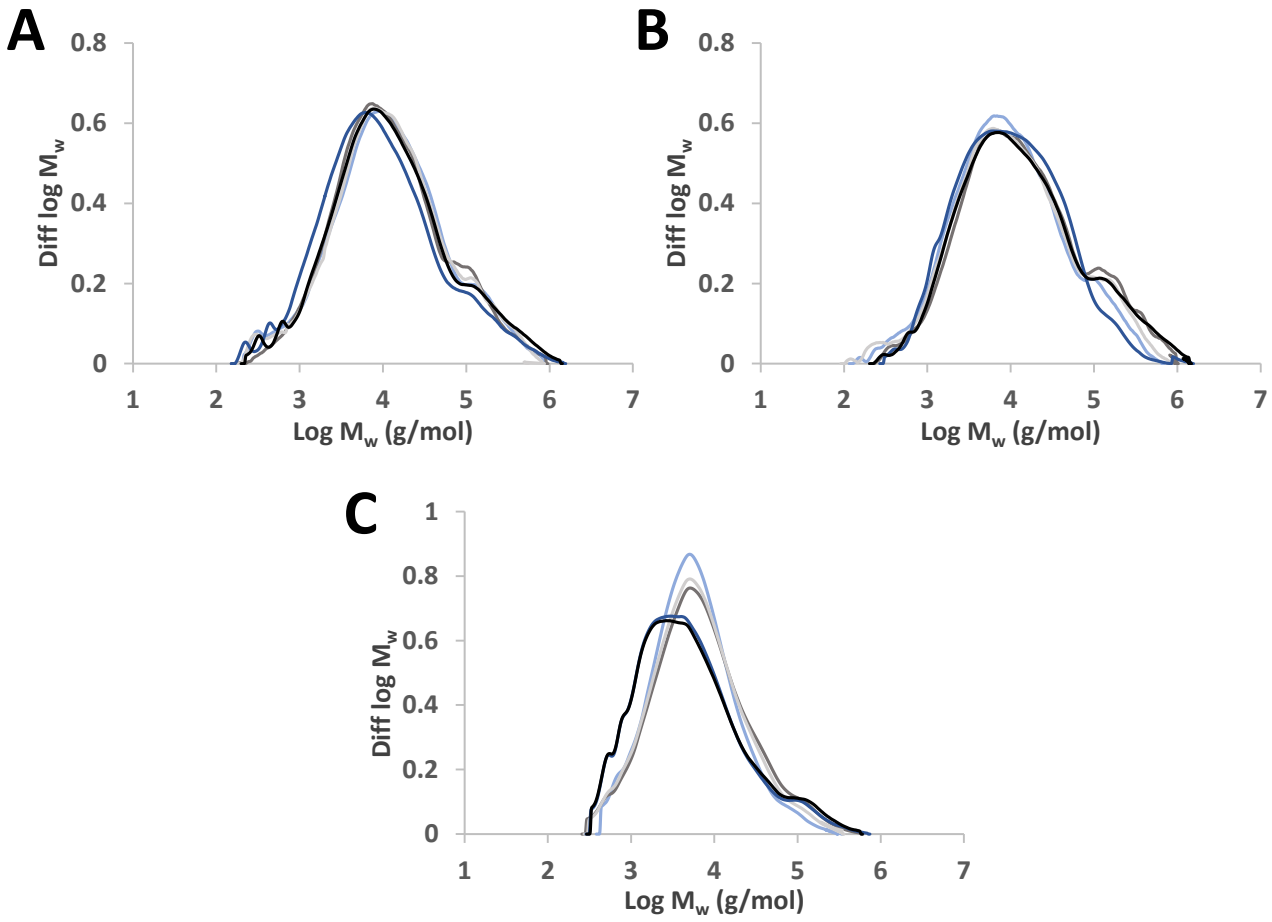

Fig. S4 Molecular weight distributions of WS CEL (A) CS CEL (B) and CS $WECEL_{pure}$ fractions as determined by using $SEC\text{-}MALS_{(IR)}$. pH 4 control = dark blue; pH 4 - 50U = light blue; pH 6 control = black; pH 6 - 50U = dark grey; pH 6 - 125U = light grey.

Table S6 Summarized RP-UHPLC-MS data of $WECEL_{pure}$ samples of laccase/HBT treated samples (LMS) and controls. Several compounds were tentatively annotated as lignin-HBT adducts, based on their molecular formula (shown in bold). These structures were absent in control incubations and in the control containing only laccase and HBT.

Rt (min)	Molecular formula	Ionization	Observed/ calculated mass	mass error (ppm)	Tentative annotation	Detected in
3.84	$C_6H_5N_3O$	$[M+H]^+$	135.04333/135.04326	0.53	1-Hydroxybenzotriazole	LMS
6.41	$C_6H_5N_3$	$[M+H]^+$	119.04841/119.04835	0.55	Benzotriazole	LMS
8.33	$C_9H_8O_3$	$[M-H]^-$	164.04751/164.04735	1.03	*p*-coumaric acid	Control & LMS
9.25	**$C_{13}H_{11}N_3O_4$**	$[M+H]^+$	273.07496/273.07496	0.03	Lignin-HBT adduct	LMS
9.38	**$C_{19}H_{19}N_3O_7$**	$[M+H]^+$	401.12263/401.12230	0.83	Lignin-HBT adduct	LMS
9.76	$C_{10}H_{10}O_4$	$[M-H]^-$	194.05801/194.05791	0.54	Ferulic acid	Control & LMS
11.00	$C_{20}H_{22}O_8$	$[M+Na]^+$	390.13175/390.13147	0.68	*Unknown*	Control & LMS
11.32	**$C_{14}H_{13}N_3O_4$**	$[M+H]^+$	287.09065/287.09061	0.17	Lignin-HBT adduct	LMS
13.78	$C_{20}H_{20}O_8$	$[M+Na]^+$	388.11610/388.11582	0.68	*Unknown*	Control & LMS
13.92	$C_{21}H_{22}O_9$	$[M+Na]^+$	418.12650/418.12639	0.26	*Unknown*	Control & LMS
14.01	**$C_{19}H_{19}N_3O_6$**	$[M+H]^+$	385.12752/385.12739	0.36	Lignin-HBT adduct	LMS
15.31	**$C_{13}H_{11}N_3O_3$**	$[M+H]^+$	257.08018/257.08004	0.55	Lignin-HBT adduct	LMS
15.86	$C_{19}H_{18}O_6$	$[M+H]^+$	342.11043/342.11034	0.28	*Unknown*	Control & LMS
17.01	$C_{20}H_{18}O_{10}$	$[M+H]^+$	418.09022/418.09000	0.54	*Unknown*	Control & LMS
18.54	$C_{17}H_{14}O_7$	$[M+H]^+$	330.07399/330.07396	0.12	Tricin	Control & LMS
21.62	$C_{22}H_{22}O_6$	$[M+Na]^+$	382.14158/382.14164	-0.15	*Unknown*	LMS

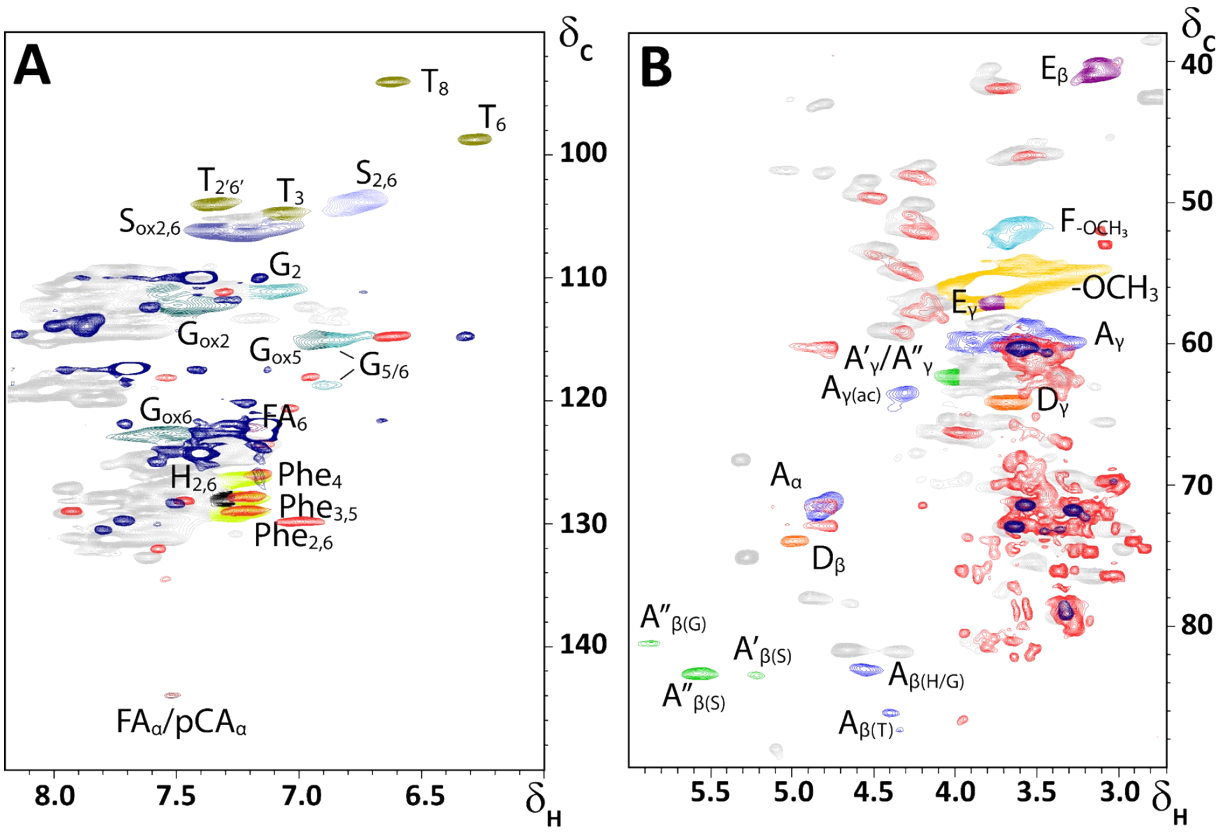

Fig. S5 Overlay of aromatic (A) and aliphatic (B) regions of the HSQC spectra obtained from the WEL_{pure} fraction of laccase/HBT treated MWS (pH 4), the Rovabio Advance enzyme cocktail, and an incubation with only laccase and HBT. Colors and annotations of the WEL_{pure} fraction are as displayed in Fig 5. Correlations from the Rovabio enzyme cocktail are shown in red and correlations from laccase+HBT are shown in navy.

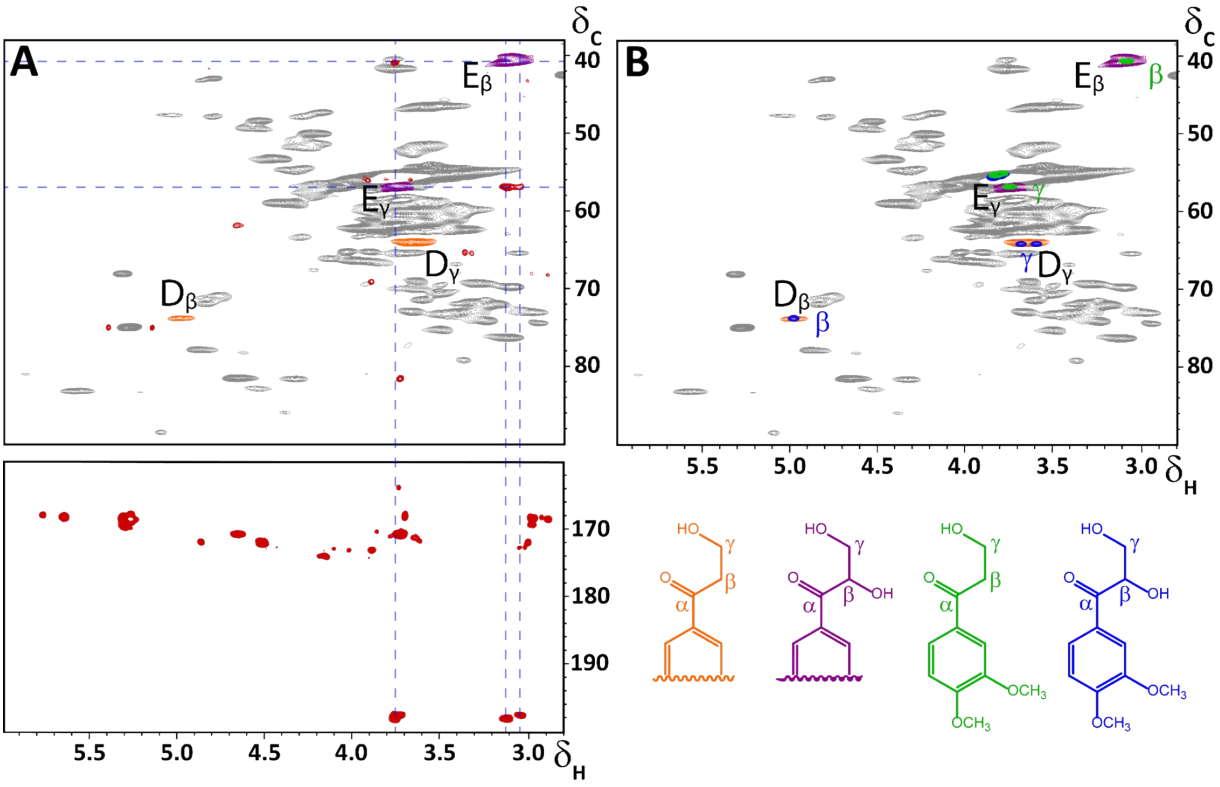

Fig. S6 NMR spectra used for the annotation of ether cleavage structures D (yellow) and E (purple): diagnostic HSQC and HMBC correlations in WEL_{pure} fraction of laccase/HBT treated MWS (pH 6, 125 U) between α, β and γ positions of E (A), and overlapping HSQC signals of D and E with purified 1-(3,4-dimethoxyphenyl)-3-hydroxypropan-1-one (green) and 1-(3,4-dimethoxyphenyl)-2,3-dihydroxypropan-1-one (blue), respectively. Due to low intensity, diagnostic HMBC correlations of structure D are not visible in (A).

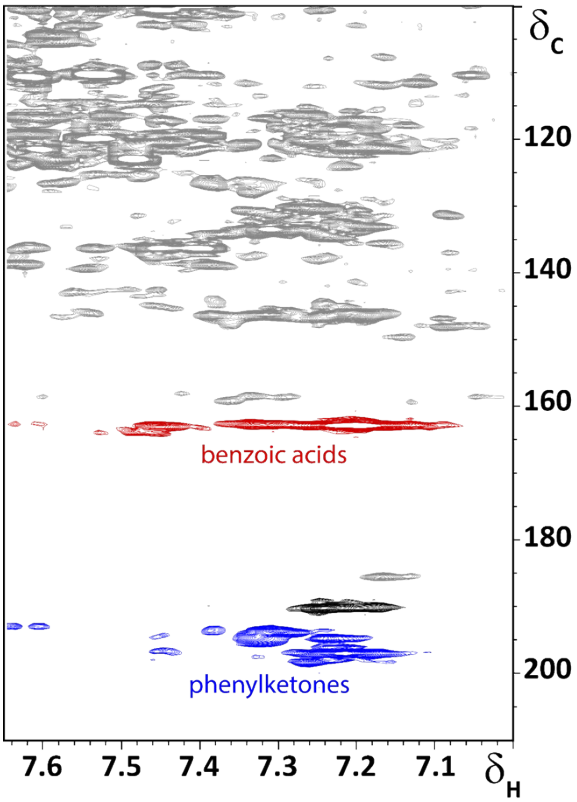

Fig. S7 HMBC spectrum of WEL_{pure} fraction of laccase/HBT treated MWS at pH 4. The spectrum is identical to the spectrum in Fig. 6C, but zoomed in (3×). The spectrum shows that a large variety of phenylketones are present, mainly corresponding to S-units. The black peak most likely corresponds to an aldehyde.

Table S7 Structural features of lignin in WEL_{pure} fractions after laccase/HBT and control treatments as measured by HSQC NMR.

	Wheat straw			**Corn stover**		
	pH 4	pH 6		pH 4	pH 6	
Laccase (U g^{-1})	50	50	125	50	50	125
Lignin subunits (%)						
H	0	0	0	0	0	0
G	14	15	11	14	14	12
G_{ox}	41	40	43	36	41	42
S	16	15	14	29	17	17
S_{ox}	30	30	32	21	29	28
S/G	0.84	0.83	0.86	1.02	0.83	0.84
Hydroxycinnamates[a]						
*p*CA	0	0	0	0	0	0
FA	0	0	0	0	0	0
Flavonoids[a]						
Tricin	8	1	2	6	1	1
Interunit linkages[a,b]						
β-*O*-4' (A)	7.5 (49)	7.0 (63)	5.1 (37)	12.1 (84)	8.6 (84)	3.7 (46)
β-*O*-4'$_{ox}$ (A'/A")	7.9 (51)	4.1 (37)	8.6 (63)	2.4 (16)	1.6 (16)	4.4 (56)
β-5' (B)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
β-β' (C)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (100%)	15.4	11.1	13.7	14.5	10.2	8.0
Cleavage products[a]						
DHPV/DHPS (D)	5.1	2.2	3.6	1.6	1.1	1.4
HPV/HPS (E)	14.6	18.7	24.7	14.5	13.6	22.5

[a] Amount of substructures per 100 aromatic subunits ($S+S_{ox}+G+G_{ox}+H$). [b] numbers between brackets refer to relative abundances of linkages.

Table S8 lignin content and relative abundances of structural features in MWS/MCS as determined by quantitative py-GC-MS.

	MWS	MCS
Lignin % (w/w)[a]	18.3 ± 0.2	22.7 ± 0.1
Lignin subunits (%)[b]		
H	3.6 ± 0.1	10.3 ± 0.2
G	56.4 ± 0.4	51.7 ± 0.6
S	40.0 ± 0.5	37.9 ± 0.4
S/G	0.7 ± 0.0	0.7 ± 0.0
Structural features (%)[b]		
Unsubstituted	10.1 ± 0.7	18.0 ± 0.2
Methyl	5.5 ± 0.4	10.2 ± 0.7
Cα-O	6.3 ± 0.3	11.2 ± 0.2
Cα-O, G	3.4 ± 0.2	6.5 ± 0.1
Cα-O, S	2.8 ± 0.1	3.3 ± 0.1
Diketones	0.8 ± 0.0	0.7 ± 0.0
Vinyl ketones	0.2 ± 0.0	0.2 ± 0.0
Cβ-O[c]	1.8 ± 0.0	2.1 ± 0.0
Cγ-O	64.2 ± 0.4	38.3 ± 1.5
Misc	6.1 ± 0.4	11.2 ± 0.9
Vinyl	6.0 ± 0.2	8.9 ± 0.3
PhCγ[d]	71.1 ± 0.0	50.5 ± 0.7
PhCγ-corrected[e]	70.0 ± 0.0	49.5 ± 0.7
PhCγ-corrected, G	42.4 ± 0.0	31.2 ± 0.7
PhCγ-corrected, S	27.5 ± 0.0	18.1 ± 0.1

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] 4-vinylphenol and 4-vinylguaicol not included in processing. [c] Excluding diketones. [d] phenols with intact 3-carbon (α, β, γ) side chain. [e] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S9 Lignin content, recovery and relative abundances of structural features in WS-RES as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	18.6±0.8	9.4±0.8	18.0±0.5	10.8±0.8	10.3±0.6
Lignin (mg)[a]	270.5±11.5	134.1±11.1	261.2±7.1	152.9±11.6	145.4±8.6
Lignin recovery vs MWS (%)[a]	98.2±4.3	48.8±4.1	94.8±2.7	55.6±4.2	52.9±3.2
Lignin subunits (%)[b]					
H	3.5 ± 0.1	7.6 ± 0.2	3.9 ± 0.1	7.2 ± 0.1	7.4 ± 0.1
G	55.8 ± 0.3	44.4 ± 0.5	56.7 ± 0.3	48.3 ± 0.6	48.4 ± 0.3
S	40.7 ± 0.4	48.0 ± 0.4	39.4 ± 0.3	44.5 ± 0.5	44.2 ± 0.2
S/G	0.7 ± 0.0	1.1 ± 0.0	0.7 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Structural moieties (%)[b]					
Unsubstituted	11.0 ± 0.9	20.8 ± 1.1	10.6 ± 0.2	17.6 ± 0.5	18.3 ± 0.4
Methyl	5.2 ± 0.5	6.9 ± 0.3	5.5 ± 0.1	8.0 ± 0.1	8.3 ± 0.1
C*α*-O	5.7 ± 0.2	16.8 ± 1.1	6.0 ± 0.1	12.4 ± 0.3	12.9 ± 0.5
C*α*-O, G	2.9 ± 0.2	8.0 ± 0.5	3.2 ± 0.0	6.0 ± 0.2	6.3 ± 0.2
C*α*-O, S	2.6 ± 0.0	8.5 ± 0.6	2.6 ± 0.0	6.1 ± 0.1	6.3 ± 0.3
Diketones	0.5 ± 0.0	4.5 ± 0.5	0.5 ± 0.0	2.1 ± 0.1	2.3 ± 0.2
Diketones, G	0.2 ± 0.0	2.2 ± 0.2	0.2 ± 0.0	0.9 ± 0.0	1.1 ± 0.1
Diketones, S	0.3 ± 0.0	2.4 ± 0.3	0.2 ± 0.0	1.2 ± 0.1	1.3 ± 0.1
Vinyl ketones	0.2 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
Vinyl ketones, G	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C*β*-O[c]	1.8 ± 0.1	2.6 ± 0.1	1.8 ± 0.0	2.6 ± 0.0	2.6 ± 0.1
C*γ*-O	64.7 ± 0.7	40.3 ± 1.6	64.9 ± 0.4	45.3 ± 0.3	43.7 ± 0.6
Miscellaneous	5.8 ± 0.5	5.8 ± 0.4	5.7 ± 0.2	7.3 ± 0.2	7.6 ± 0.3
Vinyl	5.8 ± 0.1	6.8 ± 0.1	5.5 ± 0.1	6.8 ± 0.1	6.7 ± 0.1
PhC*γ*[d]	72.0 ± 0.6	53.1 ± 1.3	71.9 ± 0.3	56.3 ± 0.5	55.2 ± 0.6
PhC*γ*-corrected[e]	71.3 ± 0.6	47.9 ± 1.8	71.3 ± 0.3	53.7 ± 0.5	52.4 ± 0.7
PhC*γ*-corrected, G	43.0 ± 0.5	22.6 ± 1.3	43.8 ± 0.2	29.1 ± 0.7	28.4 ± 0.6
PhC*γ*-corrected, S	28.2 ± 0.3	25.1 ± 0.5	27.4 ± 0.3	24.4 ± 0.3	23.9 ± 0.2

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] 4-vinylphenol and 4-vinylguaiacol not included in processing. [c] excluding diketones. [d] phenols with intact 3-carbon (α, β, γ) side chain. [e] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S10 Lignin content, recovery and relative abundances of structural features in CS-RES as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	24.8±1.0	14.8±0.7	24.4±0.6	15.9±1.0	13.8±1.3
Lignin (mg)[a]	348.6±13.7	207.9±9.4	340.6±8.4	222.2±13.9	192.2±17.9
Lignin recovery vs MCS (%)[a]	102.3±4.1	60.9±2.8	99.9±2.6	65.2±4.1	56.3±5.3
Lignin subunits (%)[b]					
H	9.2 ± 0.4	16.2 ± 0.8	10.3 ± 0.1	16.9 ± 0.4	17.4 ± 0.3
G	52.7 ± 0.3	37.5 ± 1.5	51.7 ± 0.1	40.9 ± 0.1	39.8 ± 1.0
S	38.1 ± 0.2	46.4 ± 0.8	38.0 ± 0.1	42.3 ± 0.4	42.7 ± 0.7
S/G	0.7 ± 0.0	1.2 ± 0.1	0.7 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
Structural moieties (%)[b]					
Unsubstituted	17.2 ± 0.3	25.9 ± 0.8	18.2 ± 0.4	25.7 ± 0.3	26.5 ± 1.1
Methyl	7.0 ± 0.4	8.7 ± 0.1	7.3 ± 0.2	10.2 ± 0.2	10.8 ± 0.1
Cα-O	10.2 ± 0.1	15.4 ± 0.5	10.0 ± 0.2	12.8 ± 0.3	13.6 ± 0.1
Cα-O, G	5.8 ± 0.0	6.3 ± 0.1	5.9 ± 0.1	6.1 ± 0.2	6.3 ± 0.1
Cα-O, S	3.1 ± 0.1	7.4 ± 0.4	2.7 ± 0.1	5.2 ± 0.1	5.8 ± 0.2
Diketones	0.7 ± 0.0	3.0 ± 0.3	0.5 ± 0.0	1.5 ± 0.0	1.8 ± 0.1
Diketones, G	0.2 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
Diketones, S	0.5 ± 0.0	2.2 ± 0.2	0.4 ± 0.0	1.1 ± 0.0	1.3 ± 0.0
Vinyl ketones	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Vinyl ketones, G	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Vinyl ketones, S	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[c]	2.1 ± 0.0	2.6 ± 0.0	1.7 ± 0.0	2.3 ± 0.0	2.3 ± 0.1
Cγ-O	47.6 ± 0.9	31.1 ± 1.3	48.1 ± 0.2	32.7 ± 0.6	30.3 ± 1.3
Miscellaneous	8.4 ± 0.3	8.1 ± 0.1	7.3 ± 0.2	8.3 ± 0.4	8.6 ± 0.1
Vinyl	7.4 ± 0.1	8.2 ± 0.1	7.4 ± 0.1	8.1 ± 0.1	7.9 ± 0.2
PhCγ[d]	57.6 ± 0.7	43.9 ± 1.0	56.3 ± 0.1	43.2 ± 0.4	41.4 ± 1.2
PhCγ-corrected[e]	56.7 ± 0.7	40.5 ± 1.4	55.6 ± 0.1	41.4 ± 0.4	39.3 ± 1.3
PhCγ-corrected, G	35.2 ± 0.5	18.3 ± 1.4	33.7 ± 0.1	21.0 ± 0.2	19.3 ± 1.1
PhCγ-corrected, S	21.3 ± 0.2	21.9 ± 0.1	21.8 ± 0.1	20.2 ± 0.4	19.8 ± 0.2

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] 4-vinylphenol and 4-vinylguaiacol not included in processing. [c] excluding diketones. [d] phenols with intact 3-carbon (α, β, γ) side chain. [e] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S11 Lignin content, recovery and relative abundances of structural features in WS-CEL as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	39.4 ± 5.1	23.0 ± 1.6	48.4 ± 4.0	28.7 ± 1.1	26.6 ± 0.9
Lignin (mg)[a]	81.9 ± 12.0	26.2 ± 1.9	103.0 ± 8.7	38.9 ± 1.5	33.7 ± 3.7
Lignin recovery vs MWS (%)[a,b]	66.5 ± 9.8	21.0 ± 1.5	83.2 ± 7.1	30.9 ± 1.2	26.7 ± 2.9
vs WS-RES (%)[a,b]	67.7 ± 10.3	43.0 ± 4.7	87.7 ± 7.8	55.6 ± 4.7	50.4 ± 6.2
Lignin subunits (%)[c]					
H	3.1 ± 0.4	4.6 ± 0.3	3.0 ± 0.2	3.8 ± 0.1	3.8 ± 0.1
G	54.7 ± 0.2	48.7 ± 0.6	54.3 ± 0.4	50.5 ± 0.5	50.7 ± 0.5
S	42.2 ± 0.3	46.6 ± 0.4	42.8 ± 0.3	45.7 ± 0.5	45.5 ± 0.3
S/G	0.8 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Structural moieties (%)[c]					
Unsubstituted	8.5 ± 0.2	12.9 ± 0.8	8.9 ± 0.6	10.7 ± 0.6	10.7 ± 0.3
Methyl	5.3 ± 0.5	5.6 ± 0.2	5.0 ± 0.4	5.4 ± 0.2	5.4 ± 0.2
Cα-O	5.5 ± 0.2	10.4 ± 0.1	5.4 ± 0.1	7.4 ± 0.1	7.3 ± 0.1
Cα-O, G	2.6 ± 0.1	4.9 ± 0.1	2.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.1
Cα-O, S	2.7 ± 0.1	5.2 ± 0.1	2.7 ± 0.1	3.8 ± 0.0	3.8 ± 0.0
Diketones	0.5 ± 0.0	2.1 ± 0.0	0.5 ± 0.0	1.1 ± 0.0	1.1 ± 0.0
Diketones, G	0.2 ± 0.0	1.0 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Diketones, S	0.3 ± 0.0	1.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Vinyl ketones	0.3 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Vinyl ketones, G	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[d]	1.6 ± 0.0	2.1 ± 0.1	1.6 ± 0.0	1.8 ± 0.1	1.8 ± 0.0
Cγ-O	67.4 ± 1.6	56.8 ± 1.4	67.4 ± 1.9	62.2 ± 0.5	62.4 ± 0.5
Miscellaneous	6.5 ± 0.6	5.8 ± 0.3	6.4 ± 0.6	6.1 ± 0.2	6.0 ± 0.2
Vinyl	5.2 ± 0.1	6.5 ± 0.3	5.4 ± 0.3	6.3 ± 0.2	6.3 ± 0.2
PhCγ[e]	75.1 ± 1.0	66.6 ± 1.1	75.1 ± 1.4	70.7 ± 0.5	70.9 ± 0.4
PhCγ-corrected[f]	74.4 ± 1.0	64.0 ± 1.1	74.4 ± 1.4	69.3 ± 0.5	69.4 ± 0.3
PhCγ-corrected, G	43.7 ± 0.5	33.8 ± 0.8	43.3 ± 0.9	38.0 ± 0.6	38.2 ± 0.5
PhCγ-corrected, S	30.5 ± 0.6	30.0 ± 0.4	31.0 ± 0.7	31.2 ± 0.3	31.1 ± 0.4

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] note that CEL was prepared from ~650 mg of RES. [c] 4-vinylphenol and 4-vinylguaiacol not included in processing. [d] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S12 Lignin content, recovery and relative abundances of structural features in CS-CEL as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	49.5 ± 1.9	32.6 ± 1.9	51.6 ± 4.4	32.9 ± 4.1	31.1 ± 2.0
Lignin (mg)[a]	75.9 ± 3.2	36.9 ± 3.0	80.7 ± 7.4	48.1 ± 6.1	42.4 ± 2.9
Lignin recovery vs MCS (%)[a,b]	56.8 ± 2.4	27.6 ± 2.2	60.0 ± 5.5	35.8 ± 4.5	31.4 ± 2.1
vs CS-RES[a,b]	55.5 ± 3.2	45.4 ± 4.2	60.1 ± 5.7	54.9 ± 7.7	55.8 ± 6.4
Lignin subunits (%)[c]					
H	8.9 ± 0.2	12.3 ± 0.4	9.1 ± 0.3	11.5 ± 0.1	11.5 ± 0.3
G	50.6 ± 0.2	41.7 ± 0.7	50.6 ± 0.3	44.4 ± 1.0	44.4 ± 0.6
S	40.4 ± 0.0	46.0 ± 0.8	40.3 ± 0.1	44.1 ± 0.9	44.1 ± 0.4
S/G	0.8 ± 0.0	1.1 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Structural moieties (%)[c]					
Unsubstituted	14.5 ± 0.4	20.2 ± 0.4	14.4 ± 0.6	18.8 ± 0.1	18.9 ± 0.5
Methyl	7.3 ± 0.1	8.1 ± 0.2	7.3 ± 0.3	7.8 ± 0.1	8.0 ± 0.2
C*α*-O	9.4 ± 0.0	13.5 ± 0.2	9.5 ± 0.1	11.7 ± 0.6	11.7 ± 0.3
C*α*-O, G	4.7 ± 0.0	5.6 ± 0.1	4.8 ± 0.0	5.2 ± 0.1	5.3 ± 0.1
C*α*-O, S	3.4 ± 0.0	6.4 ± 0.1	3.3 ± 0.1	5.2 ± 0.5	5.1 ± 0.2
Diketones	0.7 ± 0.0	2.3 ± 0.1	0.7 ± 0.0	1.5 ± 0.2	1.5 ± 0.1
Diketones, G	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Diketones, S	0.5 ± 0.0	1.7 ± 0.1	0.5 ± 0.0	1.1 ± 0.2	1.1 ± 0.1
Vinyl ketones	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Vinyl ketones, G	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Vinyl ketones, S	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C*β*-O[d]	1.9 ± 0.0	2.4 ± 0.0	1.9 ± 0.0	2.3 ± 0.0	2.3 ± 0.0
C*γ*-O	49.8 ± 0.7	39.0 ± 0.6	49.9 ± 1.3	42.8 ± 0.3	42.4 ± 1.1
Miscellaneous	9.0 ± 0.1	8.0 ± 0.1	8.8 ± 0.2	7.9 ± 0.3	8.0 ± 0.1
Vinyl	8.0 ± 0.1	8.8 ± 0.1	8.2 ± 0.2	8.7 ± 0.3	8.7 ± 0.1
PhC*γ*[e]	60.3 ± 0.6	51.0 ± 0.5	60.3 ± 1.2	53.6 ± 0.3	53.3 ± 1.0
PhC*γ*-corrected[f]	59.4 ± 0.6	48.3 ± 0.6	59.4 ± 1.2	51.8 ± 0.3	51.5 ± 1.1
PhC*γ*-corrected, G	36.3 ± 0.4	25.1 ± 0.7	36.3 ± 0.7	28.4 ± 0.8	28.1 ± 0.8
PhC*γ*-corrected, S	22.9 ± 0.3	23.0 ± 0.2	22.9 ± 0.5	23.2 ± 0.6	23.1 ± 0.4

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] note that CEL was prepared from ~550 mg of RES. [c] 4-vinylphenol and 4-vinylguaiacol not included in processing. [d] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S13 Lignin content, recovery and relative abundances of structural features in WS-WECEL as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	6.6 ± 0.7	6.5 ± 0.5	7.3 ± 0.2	7.0 ± 0.4	6.5 ± 0.3
Lignin (mg)[a]	41.6 ± 4.7	46.4 ± 3.8	46.2 ± 1.8	48.8 ± 2.9	45.8 ± 2.9
Lignin recovery vs MWS (%)[a,b]	33.8 ± 3.8	37.2 ± 3.1	37.3 ± 1.5	38.8 ± 2.4	36.2 ± 2.3
vs WS-RES[a,b]	34.4 ± 4.2	76.2 ± 8.9	39.4 ± 1.9	69.7 ± 6.7	68.5 ± 5.9
Lignin subunits (%)[c]					
H	9.1 ± 0.0	9.8 ± 0.4	8.7 ± 0.0	9.1 ± 0.3	9.3 ± 0.4
G	58.6 ± 0.0	48.6 ± 0.5	58.5 ± 0.5	50.6 ± 0.2	50.8 ± 0.8
S	32.3 ± 0.1	41.6 ± 0.6	32.8 ± 0.5	40.3 ± 0.4	39.9 ± 1.1
S/G	0.6 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
Structural moieties (%)[c]					
Unsubstituted	16.3 ± 0.2	24.2 ± 0.9	15.1 ± 0.2	21.3 ± 0.3	22.7 ± 1.3
Methyl	7.9 ± 0.1	8.8 ± 0.1	7.9 ± 0.1	9.0 ± 0.3	9.3 ± 0.3
Cα-O	6.8 ± 0.2	20.4 ± 0.1	6.5 ± 0.0	15.1 ± 0.2	16.1 ± 0.4
Cα-O, G	4.1 ± 0.2	10.5 ± 0.2	3.8 ± 0.0	7.6 ± 0.1	8.4 ± 0.3
Cα-O, S	2.5 ± 0.1	9.5 ± 0.1	2.4 ± 0.0	7.1 ± 0.1	7.5 ± 0.1
Diketones	0.5 ± 0.1	4.1 ± 0.1	0.4 ± 0.0	2.2 ± 0.1	2.7 ± 0.1
Diketones, G	0.3 ± 0.1	2.0 ± 0.1	0.2 ± 0.0	1.1 ± 0.0	1.4 ± 0.1
Diketones, S	0.3 ± 0.1	2.2 ± 0.0	0.2 ± 0.0	1.1 ± 0.1	1.4 ± 0.0
Vinyl ketones	0.2 ± 0.0	1.0 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Vinyl ketones, G	0.2 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[d]	1.4 ± 0.0	2.5 ± 0.0	1.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.1
Cγ-O	58.6 ± 0.4	34.3 ± 0.8	60.1 ± 0.2	41.3 ± 0.6	38.8 ± 2.0
Miscellaneous	5.4 ± 0.0	5.8 ± 0.0	5.3 ± 0.0	6.5 ± 0.2	6.6 ± 0.2
Vinyl	3.6 ± 0.0	4.1 ± 0.2	3.7 ± 0.1	4.4 ± 0.1	4.1 ± 0.1
PhCγ[e]	64.6 ± 0.3	46.6 ± 0.8	66.0 ± 0.2	51.6 ± 0.6	49.8 ± 1.9
PhCγ-corrected[f]	63.8 ± 0.4	41.5 ± 0.9	65.4 ± 0.2	48.8 ± 0.5	46.4 ± 1.9
PhCγ-corrected, G	41.9 ± 0.2	21.6 ± 0.4	42.7 ± 0.3	27.7 ± 0.4	26.2 ± 0.5
PhCγ-corrected, S	21.9 ± 0.2	19.8 ± 0.7	22.5 ± 0.5	21.0 ± 0.3	20.1 ± 1.6

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] note that WECEL was prepared from ~650 mg of RES. [c] 4-vinylphenol and 4-vinylguaiacol not included in processing. [d] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S14 Lignin content, recovery and relative abundances of structural features in CS-WECEL as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a]	10.6 ± 0.3	6.4 ± 0.4	9.7 ± 0.6	6.5 ± 0.6	5.3 ± 0.3
Lignin (mg)[a]	58.6 ± 2.7	36.8 ± 2.1	55.6 ± 4.3	36.2 ± 3.4	35.2 ± 3.9
Lignin recovery vs MCS (%)[a,b]	43.8 ± 2.0	27.6 ± 1.6	41.8 ± 3.3	27.1 ± 2.5	26.2 ± 2.9
vs CS-RES[a,b]	42.9 ± 2.6	45.3 ± 3.3	41.4 ± 3.4	41.4 ± 4.7	46.3 ± 6.7
Lignin subunits (%)[c]					
H	16.3 ± 1.8	24.3 ± 1.0	21.1 ± 3.8	23.9 ± 1.5	25.1 ± 5.4
G	57.4 ± 0.4	39.0 ± 0.2	54.9 ± 2.4	42.0 ± 0.4	37.8 ± 2.5
S	26.3 ± 1.4	36.7 ± 0.8	24.1 ± 1.4	34.2 ± 1.9	37.1 ± 2.9
S/G	0.5 ± 0.0	0.9 ± 0.0	0.4 ± 0.0	0.8 ± 0.1	1.0 ± 0.0
Structural moieties (%)[c]					
Unsubstituted	22.5 ± 2.9	35.5 ± 0.0	24.3 ± 3.0	30.3 ± 4.2	26.7 ± 4.0
Methyl	11.6 ± 1.0	12.4 ± 0.5	12.5 ± 1.3	13.8 ± 0.4	11.7 ± 0.0
Cα-O	11.5 ± 0.0	18.9 ± 0.5	11.1 ± 0.5	16.3 ± 0.1	16.6 ± 1.2
Cα-O, G	7.6 ± 0.0	8.4 ± 0.2	7.5 ± 0.3	7.8 ± 0.1	7.4 ± 0.5
Cα-O, S	2.5 ± 0.1	8.0 ± 0.2	2.4 ± 0.1	6.4 ± 0.2	7.4 ± 0.5
Diketones	0.6 ± 0.0	3.0 ± 0.1	0.6 ± 0.0	2.0 ± 0.0	2.2 ± 0.2
Diketones, G	0.2 ± 0.0	1.1 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.7 ± 0.1
Diketones, S	0.6 ± 0.0	1.9 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	1.6 ± 0.1
Vinyl ketones	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
Vinyl ketones, G	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[d]	1.2 ± 0.0	2.0 ± 0.2	1.2 ± 0.1	1.8 ± 0.0	1.9 ± 0.2
Cγ-O	43.1 ± 3.6	20.2 ± 0.2	37.2 ± 1.0	27.1 ± 5.0	28.6 ± 0.1
Miscellaneous	6.4 ± 0.0	6.9 ± 0.1	10.1 ± 4.4	6.9 ± 0.4	10.4 ± 5.8
Vinyl	3.7 ± 0.3	4.0 ± 0.2	3.5 ± 0.4	3.8 ± 0.1	4.1 ± 0.5
PhCγ[e]	49.3 ± 3.9	30.7 ± 0.1	43.4 ± 0.5	36.0 ± 5.0	37.7 ± 0.6
PhCγ-corrected[f]	48.6 ± 3.9	27.2 ± 0.0	42.6 ± 0.5	33.7 ± 4.9	34.9 ± 0.4
PhCγ-corrected, G	33.7 ± 2.3	13.7 ± 0.1	30.1 ± 0.4	18.7 ± 1.8	17.3 ± 0.2
PhCγ-corrected, S	14.7 ± 1.5	13.3 ± 0.1	12.4 ± 0.1	14.8 ± 3.1	17.5 ± 0.2

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] note that WECEL was prepared from ~550 mg of RES. [c] 4-vinylphenol and 4-vinylguaiacol not included in processing. [d] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S15 Lignin content, recovery and relative abundances of structural features in WS-WECEL$_{pure}$ as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a,b]	111.6 ± 3.8	50.4 ± 0.4	113.1[g] ± 6.2	59.7 ± 1.3	55.0 ± 1.6
Lignin (mg)[a,b]	30.5 ± 1.0	47.1 ± 0.4	46.6 ± 2.5	41.5 ± 0.9	35.9 ± 1.1
Lignin recovery vs MWS (%)[a,b,c]	24.8 ± 0.9	37.8 ± 0.5	37.6 ± 2.1	33.0 ± 0.8	28.4 ± 0.9
vs WS-WECEL[a,b,c]	84.2 ± 9.9	123.0[g] ± 10.1	115.1[g] ± 7.7	109.5[g] ± 7.0	95.9 ± 6.6
Lignin subunits (%)[d]					
H	2.1 ± 0.0	4.6 ± 0.1	2.6 ± 0.2	3.2 ± 0.1	3.6 ± 0.1
G	57.4 ± 0.3	43.8 ± 0.1	58.6 ± 0.5	46.6 ± 0.2	46.3 ± 0.4
S	40.5 ± 0.3	51.6 ± 0.0	38.7 ± 0.3	50.2 ± 0.1	50.1 ± 0.5
S/G	0.7 ± 0.0	1.2 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	1.1 ± 0.0
Structural moieties (%)[d]					
Unsubstituted	7.4 ± 0.1	19.0 ± 1.3	7.8 ± 0.1	12.6 ± 0.7	12.6 ± 0.3
Methyl	3.3 ± 0.0	5.0 ± 0.4	3.5 ± 0.2	4.5 ± 0.1	5.2 ± 0.7
Cα-O	5.8 ± 0.1	20.4 ± 0.2	5.9 ± 0.2	15.6 ± 0.2	18.4 ± 0.2
Cα-O, G	3.3 ± 0.0	8.9 ± 0.0	3.5 ± 0.2	7.1 ± 0.1	8.4 ± 0.1
Cα-O, S	2.4 ± 0.1	11.0 ± 0.2	2.2 ± 0.1	8.1 ± 0.1	9.7 ± 0.2
Diketones	0.4 ± 0.0	3.9 ± 0.1	0.4 ± 0.0	2.4 ± 0.1	3.1 ± 0.1
Diketones, G	0.2 ± 0.0	1.8 ± 0.1	0.2 ± 0.0	1.1 ± 0.0	1.3 ± 0.0
Diketones, S	0.2 ± 0.0	2.2 ± 0.1	0.2 ± 0.0	1.3 ± 0.1	1.7 ± 0.0
Vinyl ketones	0.2 ± 0.0	0.9 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.7 ± 0.1
Vinyl ketones, G	0.1 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[e]	1.4 ± 0.0	2.1 ± 0.0	1.4 ± 0.0	1.9 ± 0.0	2.1 ± 0.1
Cγ-O	75.0 ± 0.2	42.0 ± 2.3	73.9 ± 0.2	54.9 ± 1.3	51.1 ± 0.6
Miscellaneous	3.8 ± 0.0	5.3 ± 0.3	4.2 ± 0.2	5.0 ± 0.2	5.1 ± 0.2
Vinyl	3.3 ± 0.0	6.1 ± 0.1	3.2 ± 0.0	5.4 ± 0.2	5.5 ± 0.1
PhCγ[f]	79.6 ± 0.1	54.2 ± 1.8	78.7 ± 0.2	63.8 ± 1.1	61.0 ± 0.2
PhCγ-corrected[g]	79.1 ± 0.1	49.4 ± 1.9	78.1 ± 0.2	60.8 ± 1.1	57.2 ± 0.4
PhCγ-corrected, G	46.6 ± 0.1	21.9 ± 1.1	47.1 ± 0.2	29.6 ± 0.6	27.5 ± 0.1
PhCγ-corrected, S	32.4 ± 0.2	27.4 ± 0.8	30.9 ± 0.3	31.1 ± 0.5	29.6 ± 0.3

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] these numbers are expected to be an overestimation due to the high abundance of free ferulic acid (released upon XLA treatment), which is known to be pyrolzed with high efficiency.[15] [c] note that WECEL$_{pure}$ was prepared from 550-600 mg WECEL [d] 4-vinylphenol and 4-vinylguaiacol not included in processing. [e] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S16 Lignin content, recovery and relative abundances of structural features in CS-WECEL$_{pure}$ as determined by quantitative py-GC-MS.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Lignin % (w/w)[a,b]	118.1 ± 8.8	56.4 ± 1.1	121.0 ± 6.1	59.4 ± 3.5	62.4 ± 0.6
Lignin (mg)[a,b]	68.6 ± 5.1	21.3 ± 0.4	63.5 ± 3.2	22.9 ± 1.4	26.1 ± 0.3
Lignin recovery vs MCS (%)[a,b,c]	51.4 ± 3.9	15.9 ± 0.3	47.7 ± 2.4	17.1 ± 1.0	19.4 ± 0.2
vs CS-WECEL[a,b,c]	137.9 ± 12.1	71.9 ± 4.3	137.7 ± 12.7	76.7 ± 8.5	92.0 ± 10.3
Lignin subunits (%)[d]					
H	5.5 ± 0.1	10.6 ± 0.4	5.5 ± 0.3	10.1 ± 0.2	9.7 ± 0.3
G	59.1 ± 0.5	39.2 ± 0.0	59.0 ± 0.2	44.7 ± 0.3	42.5 ± 0.5
S	35.5 ± 0.6	50.2 ± 0.4	35.5 ± 0.1	45.2 ± 0.4	47.8 ± 0.2
S/G	0.6 ± 0.0	1.3 ± 0.0	0.6 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
Structural moieties (%)[d]					
Unsubstituted	12.6 ± 0.2	16.9 ± 0.5	12.7 ± 0.2	19.5 ± 0.4	17.0 ± 0.9
Methyl	5.8 ± 0.2	6.1 ± 0.1	5.5 ± 0.5	8.0 ± 0.5	6.4 ± 0.3
Cα-O	11.7 ± 0.2	21.8 ± 0.3	10.6 ± 0.4	18.1 ± 0.4	20.0 ± 0.5
Cα-O, G	7.2 ± 0.2	8.6 ± 0.1	6.4 ± 0.4	7.9 ± 0.1	8.5 ± 0.1
Cα-O, S	3.6 ± 0.0	10.2 ± 0.2	3.3 ± 0.0	8.4 ± 0.2	9.2 ± 0.3
Diketones	0.8 ± 0.0	2.8 ± 0.1	0.7 ± 0.0	2.2 ± 0.1	2.5 ± 0.1
Diketones, G	0.3 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Diketones, S	0.5 ± 0.0	2.1 ± 0.0	0.5 ± 0.0	1.6 ± 0.0	1.8 ± 0.1
Vinyl ketones	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Vinyl ketones, G	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Vinyl ketones, S	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cβ-O[e]	1.8 ± 0.0	2.1 ± 0.0	1.8 ± 0.0	2.5 ± 0.0	2.3 ± 0.1
Cγ-O	57.9 ± 0.5	41.2 ± 1.1	58.8 ± 0.9	38.0 ± 1.0	42.3 ± 2.2
Miscellaneous	5.7 ± 0.0	6.2 ± 0.3	5.8 ± 0.2	7.5 ± 0.1	6.3 ± 0.3
Vinyl	4.4 ± 0.1	5.8 ± 0.1	4.8 ± 0.1	6.4 ± 0.1	5.8 ± 0.2
PhCγ[f]	64.7 ± 0.5	51.6 ± 1.0	65.6 ± 0.7	48.7 ± 0.9	52.3 ± 1.8
PhCγ-corrected[g]	63.7 ± 0.5	48.2 ± 1.0	64.7 ± 0.7	46.0 ± 0.9	49.3 ± 2.0
PhCγ-corrected, G	40.5 ± 0.2	20.3 ± 0.3	41.8 ± 0.8	23.1 ± 0.5	22.8 ± 1.2
PhCγ-corrected, S	23.0 ± 0.5	27.6 ± 0.7	22.8 ± 0.1	22.6 ± 0.4	26.3 ± 0.7

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] these numbers are expected to be an overestimation due to the high abundance of free ferulic acid (released upon XLA treatment), which is known to be pyrolzed with high efficiency.[15] [c] note that WECEL$_{pure}$ was prepared from 450-550 mg WECEL [d] 4-vinylphenol and 4-vinylguaiacol not included in processing. [e] excluding diketones. [e] phenols with intact 3-carbon (α, β, γ) side chain. [f] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S17 Lignin content, recovery and relative abundances of structural features in WS-WEL$_{pure}$ and CS-WEL$_{pure}$ as determined by quantitative py-GC-MS.

	Wheat straw			Corn stover		
	pH 4	pH 6		pH 4	pH 6	
Laccase (U g^{-1})	50	50	125	50	50	125
Lignin % (w/w)[a]	23.2 ± 1.9	6.6 ± 0.4	15.4 ± 0.6	20.9 ± 3.2	9.1 ± 0.3	12.7 ± 0.3
Lignin (mg)[a,b]	6.9 ± 0.6	4.6 ± 0.3	5.7 ± 0.2	3.6 ± 0.6	2.6 ± 0.1	2.9 ± 0.1
Lignin recovery vs MWS/MCS (%)[b,c,d]	5.0 ± 0.4	3.4 ± 0.2	4.2 ± 0.2	2.1 ± 0.3	1.5 ± 0.0	1.7 ± 0.0
vs WS/CS-WEL (%)[b,c,d]	9.7 ± 1.8	7.6 ± 1.6	8.8 ± 1.3	5.4 ± 1.1	4.4 ± 1.0	4.0 ± 1.0
Lignin subunits (%)[d]						
H	5.4 ± 0.0	9.7 ± 0.2	5.4 ± 0.1	12.2 ± 0.0	15.6 ± 0.5	13.4 ± 1.5
G	43.8 ± 0.3	40.5 ± 0.0	42.9 ± 0.3	33.9 ± 0.3	32.9 ± 0.0	31.7 ± 0.9
S	50.8 ± 0.3	49.9 ± 0.2	51.7 ± 0.4	53.9 ± 0.3	51.5 ± 0.5	54.9 ± 0.9
S/G	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0
Structural moieties (%)[d]						
Unsubstituted	16.2 ± 1.9	21.9 ± 0.2	17.5 ± 0.5	18.5 ± 0.1	31.2 ± 1.3	22.9 ± 0.9
Methyl	3.0 ± 0.1	3.4 ± 0.1	3.0 ± 0.1	4.3 ± 0.0	6.1 ± 0.2	4.7 ± 0.4
Cα-O	33.5 ± 1.1	37.1 ± 0.0	34.4 ± 0.5	32.2 ± 0.2	35.6 ± 0.6	36.5 ± 0.8
Cα-O, G	15.8 ± 0.4	15.8 ± 0.4	15.4 ± 0.3	13.6 ± 0.1	12.6 ± 0.3	12.6 ± 0.2
Cα-O, S	17.2 ± 0.6	18.1 ± 0.2	18.4 ± 0.3	16.2 ± 0.2	20.9 ± 0.4	21.9 ± 0.7
Diketones	8.3 ± 0.6	7.3 ± 0.5	6.8 ± 0.2	5.2 ± 0.2	7.2 ± 0.5	7.1 ± 0.2
Diketones, G	3.6 ± 0.3	3.5 ± 0.1	2.8 ± 0.2	1.5 ± 0.2	2.2 ± 0.2	2.2 ± 0.2
Diketones, S	4.7 ± 0.3	3.9 ± 0.3	3.9 ± 0.1	3.7 ± 0.1	5.0 ± 0.2	5.0 ± 0.2
Vinyl ketones	2.6 ± 0.0	2.3 ± 0.4	2.6 ± 0.1	1.3 ± 0.0	1.7 ± 0.1	2.1 ± 0.0
Vinyl ketones, G	2.1 ± 0.0	1.8 ± 0.0	2.1 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.5 ± 0.0
Vinyl ketones, S	0.6 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
Cβ-O[e]	1.6 ± 0.0	2.0 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	2.3 ± 0.0	2.1 ± 0.0
Cγ-O	40.3 ± 1.2	30.5 ± 0.2	37.8 ± 0.8	34.9 ± 0.3	17.5 ± 0.8	25.9 ± 1.0
Miscellaneous	2.2 ± 0.1	2.2 ± 0.0	2.4 ± 0.1	3.2 ± 0.0	3.2 ± 0.0	3.2 ± 0.0
Vinyl	3.2 ± 0.1	2.8 ± 0.0	3.2 ± 0.1	5.2 ± 0.1	4.1 ± 0.1	4.7 ± 0.1
PhCγ[f]	55.0 ± 1.7	43.8 ± 0.1	51.2 ± 0.7	46.1 ± 0.5	31.4 ± 1.3	40.2 ± 0.9
PhCγ-corrected[g]	44.1 ± 1.1	34.2 ± 0.2	41.9 ± 0.9	39.5 ± 0.2	22.5 ± 0.8	31.0 ± 1.1
PhCγ-corrected, G	20.2 ± 0.8	14.6 ± 0.4	19.2 ± 0.5	13.5 ± 0.3	8.1 ± 0.3	11.2 ± 0.9
PhCγ-corrected, S	23.8 ± 0.4	19.5 ± 0.6	22.7 ± 0.4	25.9 ± 0.2	14.3 ± 0.5	19.8 ± 0.2

[a] 4-vinylphenol and 4-vinylguaiacol included in processing. [b] note that WEL$_{pure}$ was purified from ~50% of the obtained WEL [c] the amount of lignin in WEL was calculated by subtracting the amount of lignin in PWS/PCS with that in WS/CS-RES. [d] 4-vinylphenol and 4-vinylguaiacol not included in processing. [e] excluding diketones. [f] phenols with intact 3-carbon (α, β, γ) side chain. [g] phenols with intact 3-carbon (α, β, γ) side chain minus diketones and vinylketones.

Table S18 ^{13}C-IS py-GC-MS relative abundance of 4-vinylphenol in fractions of laccase/HBT treated MWS and MCS and controls.

	pH 4		pH 6		
Laccase (U g^{-1})	0	50	0	50	125
Wheat straw					
RES	8.7 ± 0.8	8.9 ± 0.9	8.1 ± 0.9	8.2 ± 0.9	8.1 ± 0.7
CEL	8.7 ± 0.8	10.6 ± 1.1	9.4 ± 1.1	10.0 ± 1.1	10.1 ± 0.9
$WECEL_{pure}$	6.5 ± 0.5	8.5 ± 0.7	7.6 ± 0.6	9.4 ± 0.8	9.8 ± 0.8
WEL_{pure}	-	1.0 ± 0.1	-	1.0 ± 0.1	1.2 ± 0.1
Corn stover					
RES	30.6 ± 2.7	39.3 ± 3.5	31.7 ± 3.4	36.9 ± 2.9	35.4 ± 3.0
CEL	38.5 ± 3.4	44.1 ± 3.8	40.0 ± 3.3	41.3 ± 3.9	41.0 ± 3.8
$WECEL_{pure}$	16.8 ± 1.4	38.6 ± 3.1	18.4 ± 1.5	35.9 ± 3.0	31.5 ± 2.5
WEL_{pure}	-	8.7 ± 0.7	-	4.6 ± 0.4	5.0 ± 0.4

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